STUDIES IN THE DEVELOPMENT OF FROG HYBRIDS.
IV. COMPETENCE OF GASTRULA ECTODERM IN ANDROGENETIC HYBRIDS

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Hybrid embryos obtained from the fertilization of *Rana pipiens* Schreber ova with sperm of *Rana sylvatica* Le Conte develop in a seemingly normal manner to the beginning of gastrulation (Moore, 1946). In the majority of cases the hybrids form a tiny dorsal lip and differentiate no further. They remain as arrested gastrulae for nearly a week and then cytolize. The failure of further differentiation of these hybrids has been found to be correlated with reduced competence of the presumptive epidermis (Moore, 1947) and reduced inductive ability of the dorsal lip (Moore, 1948) as compared with normal embryos.

In a further effort to dissociate the factors responsible for the cessation of development in the hybrids, a study has been made of androgenetic hybrids of *R. pipiens* ♀ × *R. sylvatica* ♂ obtained by removal of the maternal chromosomes. The development of these haploid hybrids tests the ability of the *sylvatica* nucleus to influence the development of the *pipiens* egg devoid of *pipiens* chromosomes. It must not be imagined, however, that the enucleated *pipiens* ovum is merely a passive substrate for a foreign sperm to mould. We shall be studying the effect of *sylvatica* genes on the egg cytoplasm that was organized in the ovary under the influence of *pipiens* genes. There is a considerable body of evidence to indicate that the development of the egg up to late blastula or early gastrula is already determined at the time it leaves the female (Moore, 1941). In the case of these androgenetic hybrids, therefore, we should not expect an effect of the *sylvatica* chromosomes during the cleavage and blastula stages.

MATERIALS AND METHODS

Eggs were obtained from a *R. pipiens* by pituitary injections. Some were fertilized with *pipiens* sperm and others with *sylvatica* sperm. Using the method of Porter (1939), the maternal chromosomes were removed from a number of the eggs. The four classes of embryos obtained were normal *pipiens*, *pip* × *pip;* haploid *pipiens*, (*pip*) × *pip;* hybrids, *pip* × *syl;* and haploid hybrids, (*pip*) × *syl*.

The *pip* × *pip* embryos developed normally. The (*pip*) × *pip* embryos were identical with the diploid normals during the early stages. The first deviation from normality occurred during gastrulation, when there was a slight retardation in the rate of development. In later stages morphological abnormalities, characteristic of the haploid syndrome (Porter, 1939; Moore, 1950), appeared and the embryos died as edematous larvae.

The *pip* × *syl* hybrids developed normally to the beginning of gastrulation. Thereafter, development was completely abnormal (Moore, 1946). Wrinkles and

furrows formed in the blastocoel roof, which later became smooth as the entire embryo swelled. Eventually the blastocoel roof appeared to collapse and the embryo was left as a much wrinkled gastrula. Cytolysis began when the *pip* × *pip* embryos were in stage 20 (gill circulation) or 21 (cornea transparent).

The (*pip*) × *syl* embryos developed normally until the late blastula stage, as has been noted previously by Ting (1951). Epiboly was not as extensive as in *pip* × *syl* and the dorsal lip never formed. A grayish region on one side of the embryo may have been an indication of where the dorsal lip would have appeared, if differentiation had proceeded further. The (*pip*) × *syl* embryos, like the diploid hybrids, became swollen and remained alive until the normal *pipiens* controls reached stage 21. Thus, at a temperature of 20°, they remained as arrested blastulae for approximately one week. During this period the haploid hybrids appeared perfectly healthy and did not exhibit the characteristic wrinkles, pits and furrows of the diploid hybrids, nor did they shrink in size before cytolysis.

Since diploid hybrids of *pip* × *syl* have a dorsal lip, and haploid hybrids of (*pip*) × *syl* do not, it is possible to be sure of using the desired embryos for transplantation. The few embryos in the (*pip*) × *syl* group which formed a dorsal lip were assumed to be diploid, due to failure of removal of the maternal chromosomes, and were not used in the transplantation experiments. The (*pip*) × *pip* early gastrulae can be definitely distinguished from the *pip* × *pip* embryos by the slight retardation in development.

EXPERIMENTS

Competence of the gastrula ectoderm was tested by transplanting pieces of the blastocoel roof of an early gastrula to the pronephric region of older embryos, in a manner previously described (Moore, 1947). Such transplants, under the influence of the host cells, form neural tissue and other structures if competent, as Holtfreter (1933) has demonstrated. The host in all of the experiments was *Rana palustris* Le Conte. Embryos of this species are much lighter in color than *R. pipiens* embryos and, therefore, host and donor cells are readily distinguishable. This difference in pigmentation is apparent in histological preparations as well. The hosts were in stage 17 (tail-bud) at the time of transplantation. One donor contributed transplants to two hosts.

Fourteen transplants of (*pip*) × *syl* presumptive ectoderm were made and compared with similar transplants of 8 (*pip*) × *pip*, 10 *pip* × *syl* and 2 *pip* × *pip*. The (*pip*) × *pip* transplants served as the controls for haploidy, while the *pip* × *syl* experiments formed the chief basis of comparison. The behavior of *pip* × *syl* and *pip* × *pip* tissue has been studied extensively in previous experiments (Moore, 1947), so relatively few operations of this material were performed for the present experiments.

It was found that when the control *pip* × *pip* were early gastrulae, the (*pip*) × *pip* were slightly earlier gastrulae; the *pip* × *syl* showed pigment at the dorsal lip and no invagination; and the (*pip*) × *syl* were late blastulae. Since it was desirable to have the different transplants in the same relative stage of differentiation, transplants were made from *pip* × *pip*, (*pip*) × *pip* and *pip* × *syl* when they were in stage 10 (early gastrulae). Since the *pip* × *syl* are arrested at stage 10, and the formation of the dorsal lip is retarded, they were used when they had been

in this stage for a short time.   Control *pip* × *pip*, fertilized at the same time and
kept under the same conditions, were in stage 11 (semi-circular blastopore).   The
(*pip*) × *syl* had to be used in stage 9 (late blastulae), as they never form a dorsal
lip.   Since their development was retarded at this time, they were used when the
control *pip* × *pip* were in stage 12 (yolk plug).

In the transplants from the three control types, the *palustris* hosts were allowed
to develop to stage 21.   They were then fixed and studied in serial section.   In the
transplants involving (*pip*) × *syl* tissue, the hosts were fixed in stage 20, since
some of the transplants were unhealthy at that stage.

<div align="center">RESULTS</div>

The 14 transplants of (*pip*) × *syl* ectoderm showed no differentiation what-
soever.   The cells remained as large, late blastula cells.   There was little variation
among the 14 cases and Figures 1 and 2 may be taken as typical of the results.
It was noticed that the donor cells stained much less intensely than the host cells
with fast green.

The 10 *pip* × *syl* transplants showed slightly better differentiation than the
haploid hybrid transplants.   The cells were smaller in size than in the (*pip*) × *syl*
experiments.   A two-layered epidermis was formed with an underlying mass of
tissue which could not be called either neural or neuroid.   The degree of differen-
tiation was similar to that shown in Moore (1947), Figure 8.   This represents the
lowest degree of differentiation that can be expected with this tissue.

The 8 (*pip*) × *pip* transplants were distinctly better in their differentiation than
either previously mentioned class.   The transplants formed a two-layered epidermis
with underlying neuroid masses.   Figures 3 and 4 are typical.

The two *pip* × *pip* transplants showed the greatest differentiation.   As in ac-
cordance with earlier work (Moore, 1947), neural tissue was formed.

<div align="center">DISCUSSION</div>

From the data described above, it is evident that the presumptive ectoderm of
(*pip*) × *syl* is lacking in competence and, when transplanted, cannot differentiate
any further than in the entire haploid hybrid embryo.   This result throws some
light on the factors involved in the cessation of development of the diploid hybrids.
We may imagine that there are three main components in a *pipiens* ♀ × *sylvatica* ♂
hybrid, namely: *pipiens* cytoplasm, a haploid set of *pipiens* chromosomes and a
haploid set of *sylvatica* chromosomes.   When all three components are present,
normal development ceases at the beginning of gastrulation.   Since the combination
of *pipiens* cytoplasm plus a haploid set of *pipiens* chromosomes alone can produce
an embryo which reaches the larval stage of development, it is clear that the addition
of a haploid set of *sylvatica* chromosomes is having an antagonistic effect on the
functioning of the *pipiens* chromosomes, or of the *pipiens* cytoplasm or of both.
Since the development of an embryo with *pipiens* cytoplasm and a haploid set of
sylvatica chromosomes is even less after the removal of the haploid set of *pipiens*
chromosomes, it is evident that there is an antagonistic effect between the *sylvatica*
chromosomes and the *pipiens* cytoplasm.   That this is a real antagonism has been
demonstrated by the transplantation experiments, where no response can be elicited

PLATE I
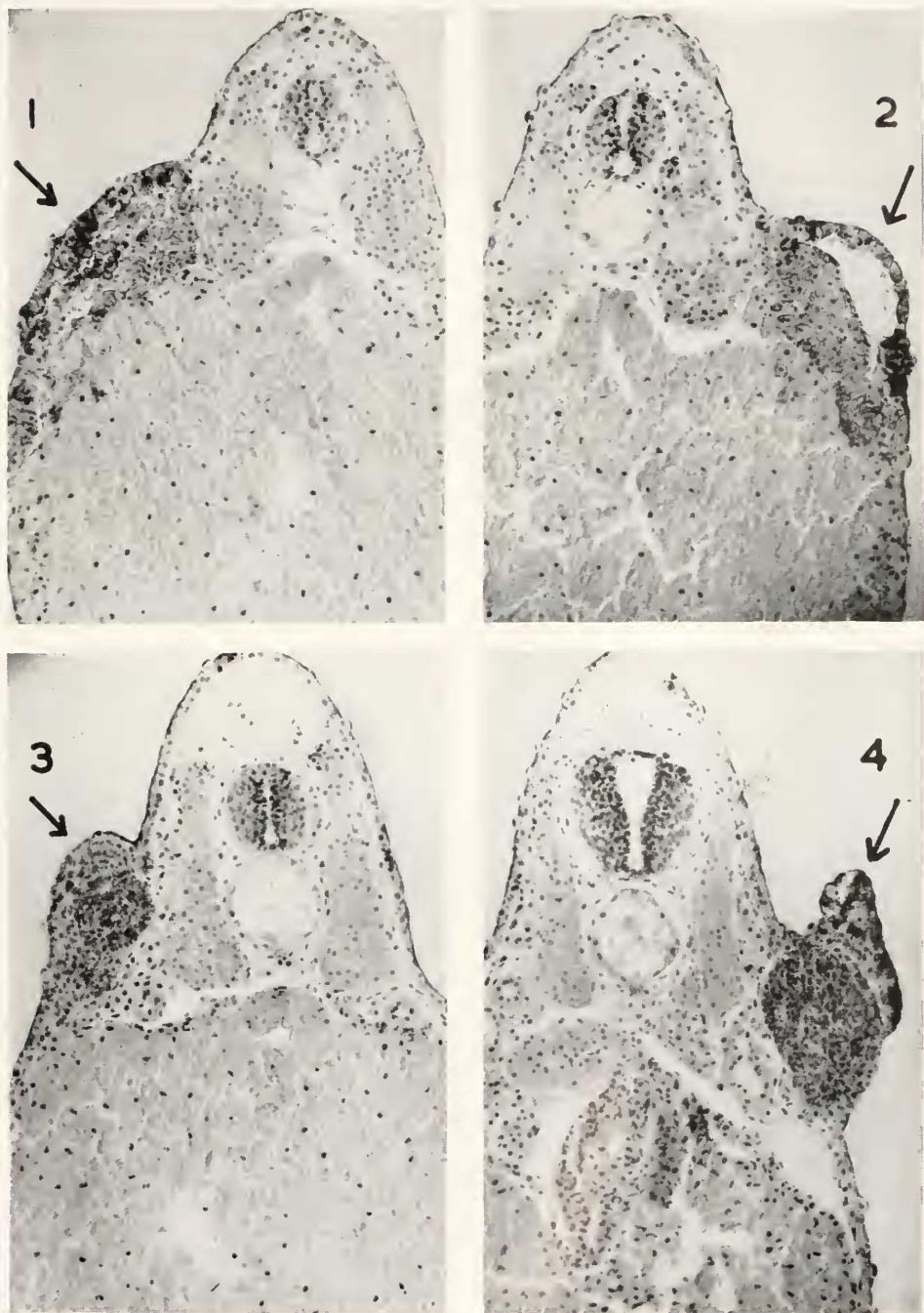
FIGURES 1 AND 2. Development of androgenetic *pipiens* ♀ × *sylvatica* ♂ gastrula ectoderm in *Rana palustris*. The region of the donor tissue is indicated by an arrow.

FIGURES 3 AND 4. Development of androgenetic *pipiens* gastrula ectoderm in *Rana palustris*. The region of the donor tissue is indicated by an arrow.

in the haploid hybrid tissue, even though in a normal environment. From these experiments it is evident that the result of the antagonistic action between the *sylvatica* chromosomes and the *pipiens* cytoplasm is actually lessened by the presence of a set of *pipiens* chromosomes, since the development of the diploid hybrid is greater than that of the haploid hybrid, and transplanted ectoderm is capable of considerably greater differentiation (Moore, 1947).

A finding of considerable interest is the evidence of a gradation in competence of the presumptive ectoderm of the various types of embryos studied. The results indicate that diploid *pipiens* presumptive ectoderm is more competent than haploid *pipiens* presumptive ectoderm. The last is more competent than the diploid hybrid presumptive ectoderm which is more competent than the haploid hybrid presumptive ectoderm.

Although only two transplants of *pip* × *pip* tissue and 8 of (*pip*) × *pip* tissue were made, in a previous experiment (Moore, 1947) 18 transplants of *pip* × *pip* gastrula ectoderm were described. In each one of these 20 transplants, neural tissue was formed from the donor cells, whereas none of the 8 (*pip*) × *pip* transplants formed good neural tissue. Their response was at a distinctly lower level of differentiation which is customarily called neuroid. More recent experiments (unpublished data) have shown that out of 33 *pip* × *pip* transplants, 91% formed neuroid or neural tissue, but such a response was shown by only 44% of 32 (*pip*) × *pip* transplants.

Similar testing of the competence of haploid tissue has not been studied by other workers. In all probability the degree of competence of such tissue will vary from species to species, even as the development of whole haploid embryos varies. An androgenetic *Triton taeniatus* has reached metamorphosis (Baltzer, 1922; Fankhauser, 1938) and obviously such haploid tissue is competent and capable of normal differentiation. Although the (*pip*) × *pip* embryos die as young larvae and the presumptive ectoderm has been shown to have reduced competence, this does not necessarily indicate that such tissue is incapable of good differentiation when transplanted to a normal diploid host. Dalton (1946) has shown that transplanted haploid neural crest cells of *Triturus rivularis* can produce the normal pigment pattern, whereas the whole merogone reaches only the tailbud stage. Hadorn (1932, 1937) has shown that haploid *Triton palmatus* tissue is capable of normal, though retarded, differentiation, in contrast to the whole merogones, which die between tailbud and forelimb stage.

The diploid hybrid, *pip* × *syl*, showed reduced competence of the presumptive ectoderm when compared with the diploid and haploid *pipiens* embryos. Histological differentiation of *pip* × *syl* tissue is poor, even when incorporated with the host neural tissue (Moore, 1947). This is in marked contrast to transplants of lethal diploid tissue described by Lüthi (1938). He found that androgenetic *Triton palmatus* ♀ × *Salamandra maculosa* ♂ gastrula tissue would develop normally when transplanted into a diploid *T. palmatus* gastrula. The whole hybrid embryo develops no further than middle gastrula stage (Schönmann, 1938).

The complete lack of differentiation of the haploid hybrid tissue, (*pip*) × *syl*, is also in marked contrast to studies made by other workers. Haploid hybrid neural crest cells of *Triturus rivularis* ♀ × *Triturus torosus* ♂ have been trans-

planted by Dalton (1946) and were found capable of producing a pigment pattern, whereas the whole merogone developed only to the tailbud stage.   De Roche (1937) found that transplanted presumptive ectoderm of androgenetic hybrid tissue between *Triton alpestris* ♀ × *Triton palmatus* ♂ developed normally, whereas whole merogones only developed as far as closed neural folds.   Since these haploid hybrids differentiate at least as far as neurulae, their developmental capabilities should be greater than (*pip*) × *syl* tissue, which does not develop to a gastrula stage.   Moreover, the diploid hybrids of these species reach metamorphosis, in contrast to the *pip* × *syl*, which die as early gastrulae.

Hadorn (1932) found that implanted androgenetic hybrid gastrula tissue of *Triton palmatus* ♀ × *Triton cristatus* ♂ formed normal tissue, except for pycnotic head mesenchyme.   The whole merogones reached closed neural folds, with pycnotic head mesenchyme.   In later experiments in which chimeras of androgenetic *T. palmatus* ♀ × *T. cristatus* ♂ gastrulae and diploid hybrid *T. palmatus* ♀ × *T. cristatus* ♂ gastrulae were studied, Hadorn (1937) found that the androgenetic hybrid tissue was incapable of differentiating anterior head structures.   Additional experiments with the androgenetic hybrid material indicated that the ectoderm is incapable of forming an optic vesicle and that there may be reduced inductive ability of the head organizer.   These latter experiments, which show a localized lack of competence, affecting anterior head structures, of (*palmatus*) × *cristatus* tissue, are of particular interest when compared with our experiments, which show no competence of (*pip*) × *syl* tissue.   We would expect the developmental capabilities of (*palmatus*) × *cristatus* tissue to be considerably greater than that of (*pip*) × *syl* tissue, since diploid hybrids of *palmatus* × *cristatus* may metamorphose and the haploid hybrids reach closed neural folds.

From this comparison of similar transplantation experiments of other workers with ours, it is evident that the degree of differentiation of such transplants varies with the species used.   When a comparison of transplants of diploid, haploid, diploid hybrid and haploid hybrid tissues, such as were described in this paper, is made, however, it is found that there is a gradation in the differentiating capabilities of such tissues, from a maximum for the diploid to none for the haploid hybrid tissue.

Summary

1. In an effort to dissociate the factors responsible for the failure of development in *Rana pipiens* ♀ × *Rana sylvatica* ♂ hybrids, a study has been made of the development of haploid embryos composed of *pipiens* cytoplasm and *sylvatica* chromosomes.   These androgenetic hybrids develop only to the late blastula stage.

2. The competence of the presumptive ectoderm of these haploid hybrids was tested by transplantation to neurulae of *Rana palustris*.   The results indicated a total lack of competence.

3. Aside from the main problem with which the paper is concerned, it was found that the various classes of embryos used could be arranged in order of decreasing competence of the presumptive ectoderm.   The sequence was as follows: diploid *pipiens*, haploid *pipiens*, *pipiens* ♀ × *sylvatica* ♂ diploid hybrids and (*pipiens* ♀) × *sylvatica* ♂ haploid hybrids.

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